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Defensin peptides, naturally occurring neutrophil antibiotics, were purified and characterized functionally and structurally. Functional differences in microbicidal potency and spectrum were correlated with structural features resolved by NMR and crystallographic methods. Protocols were established for synthesis and disulfide formation of natural defensins and synthetic congeners; we are currently investigating the latter by structural and functional characterization.

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FINAL TECHNICAL REPORT

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Defensins, homologous antimicrobial peptides isolated from neutrophils of several mammalian species, were employed in structure-activity studies aimed at the design of novel antimicrobial peptides. As defensins are small (29-34 residues) and constrained by three intramolecular disulfides, they are all thought to possess the same overall fold. Therefore, these peptides are ideally suited to SAR studies. Further, the naturally-occurring functional (microbicidal potency and spectrum) variation among members of this family provides a "head-start" in devising a scheme for dissecting structure and function.

During the term of the contract, a number of new defensins were discovered by us and others. The family of defensin peptides now known is shown in Fig. 1.

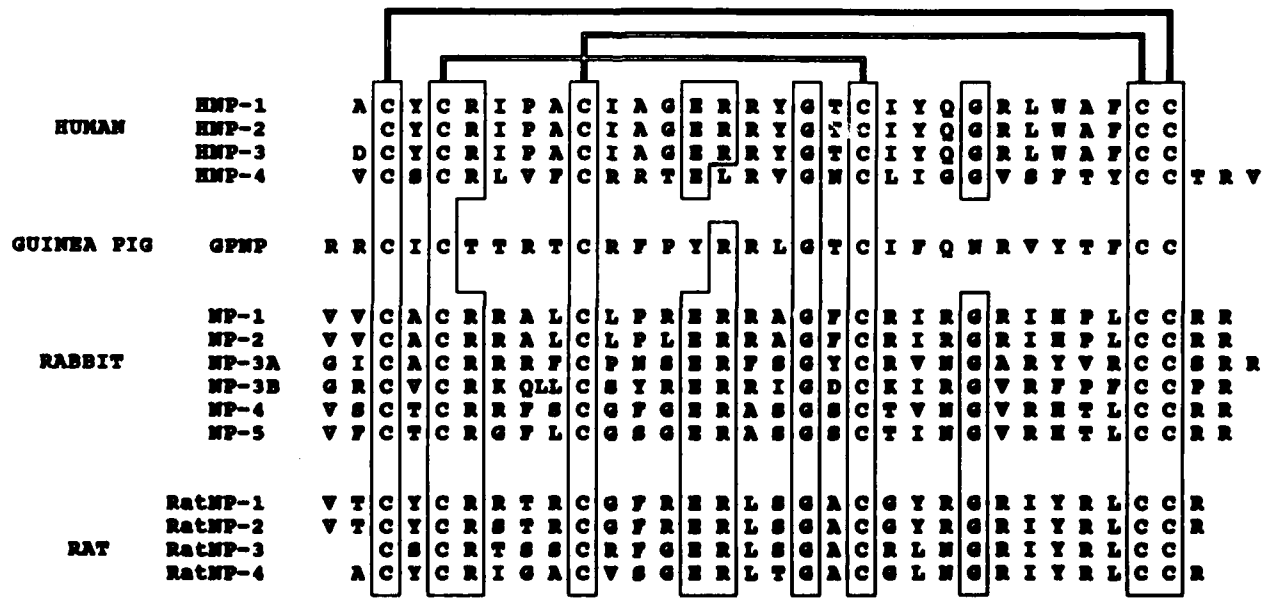

Figure 1. Amino acid sequences and disulfide connectivities of defensins.

The disulfide connectivities were determined using a novel biochemical approach, revealing that the disulfide motif in defensins is unique. Note that in HNP-2, a disulfide connects the amino and carboxyl terminal residues, a feature not known to occur in any other protein. (Fig. 2).

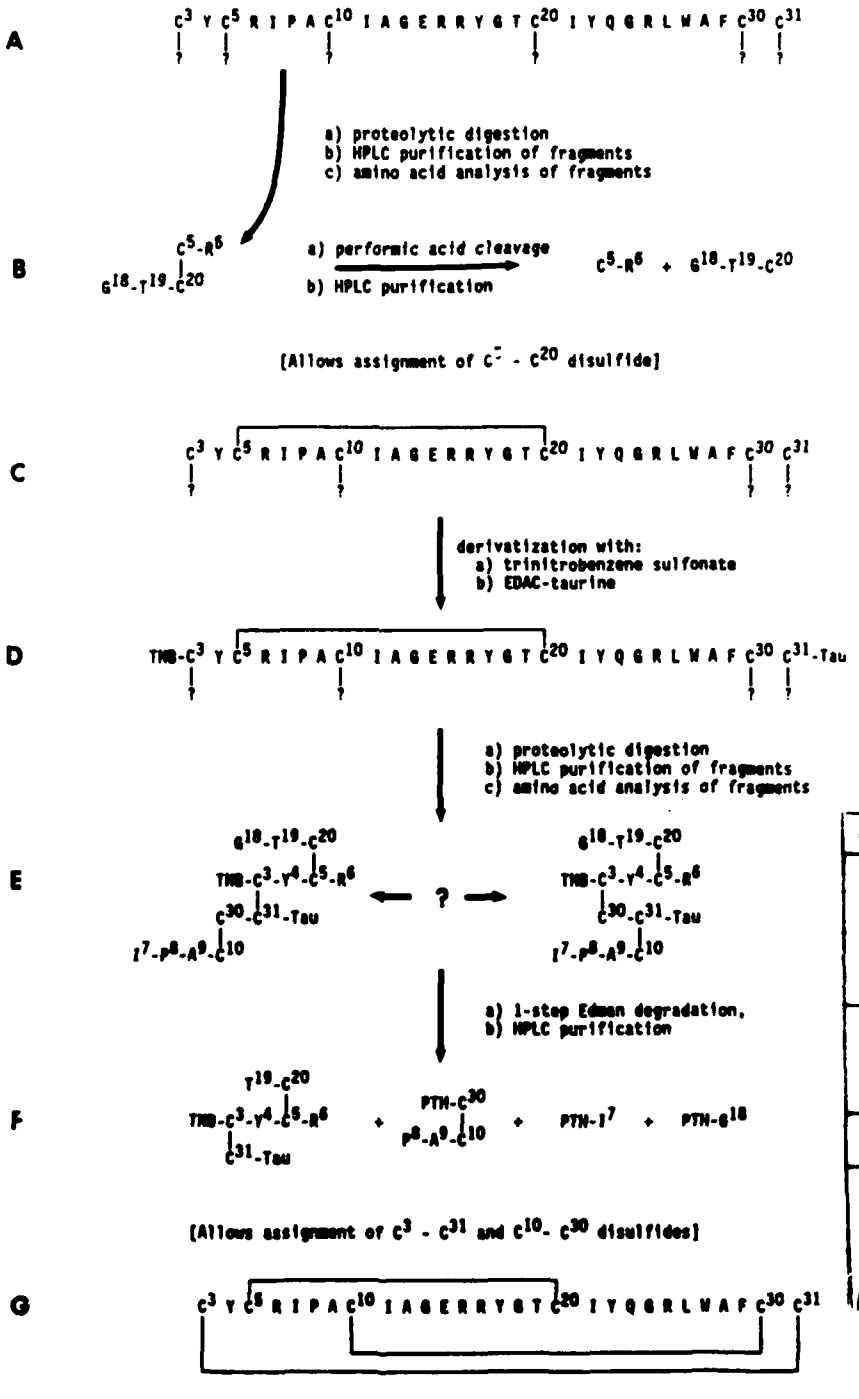

Fig. 2. Determination of the disulfide motif in defensin HNP-2.

Structural studies of defensins were carried out in solution and in crystals. Two dimensional NMR revealed that HNP-1, NP-2, and NP-5 were very similar in their overall fold, and that each forms a three stranded antiparallel sheet (Fig. 3).

Figure 3 Comparison of the backbone conformation of HNP-1, NP-2 and NP-5. The main chains of HNP-1 (red), NP-2 (yellow) and NP-5 (blue) were superimposed by calculating the best fit for C, N, O and C^{α} atoms for residues 16-31 in each peptide.

Of the several defensin peptides which have been crystallized, HNP-3 was found to be most useful for diffraction studies. Platinum salt derivatives of HNP-3 were used to solve the structure of this peptide at 1.9 A (R_{factor} = 0.19; Fig. 4). The crystal structure of HNP-3 revealed a dimeric structure of this defensin, and confirmed both the disulfide assignments and the solution conformation. The dimer of HNP-3 is amphiphilic, as there is a large patch of leucine and isoleucine residues concentrated at one pole, while the N and C termini of both chains are positioned at the opposite pole. The amphiphilic topology is thought to correlate with the ability of defensins to permeabilize lipid bilayers.

One of the major goals of the project was to utilize solid phase synthesis for producing defensin analogs. We utilized both BOC and Fmoc chemistries to synthesize NP-2, HNP-1, and GPNP. Although refolding and oxidation of each peptide required very different conditions, we were able to derive conditions for each peptide which resulted in quantitative, correct disulfide formation. The first set of synthetic congeners has been produced, and we are currently evaluating the antimicrobial activities of these analogs.

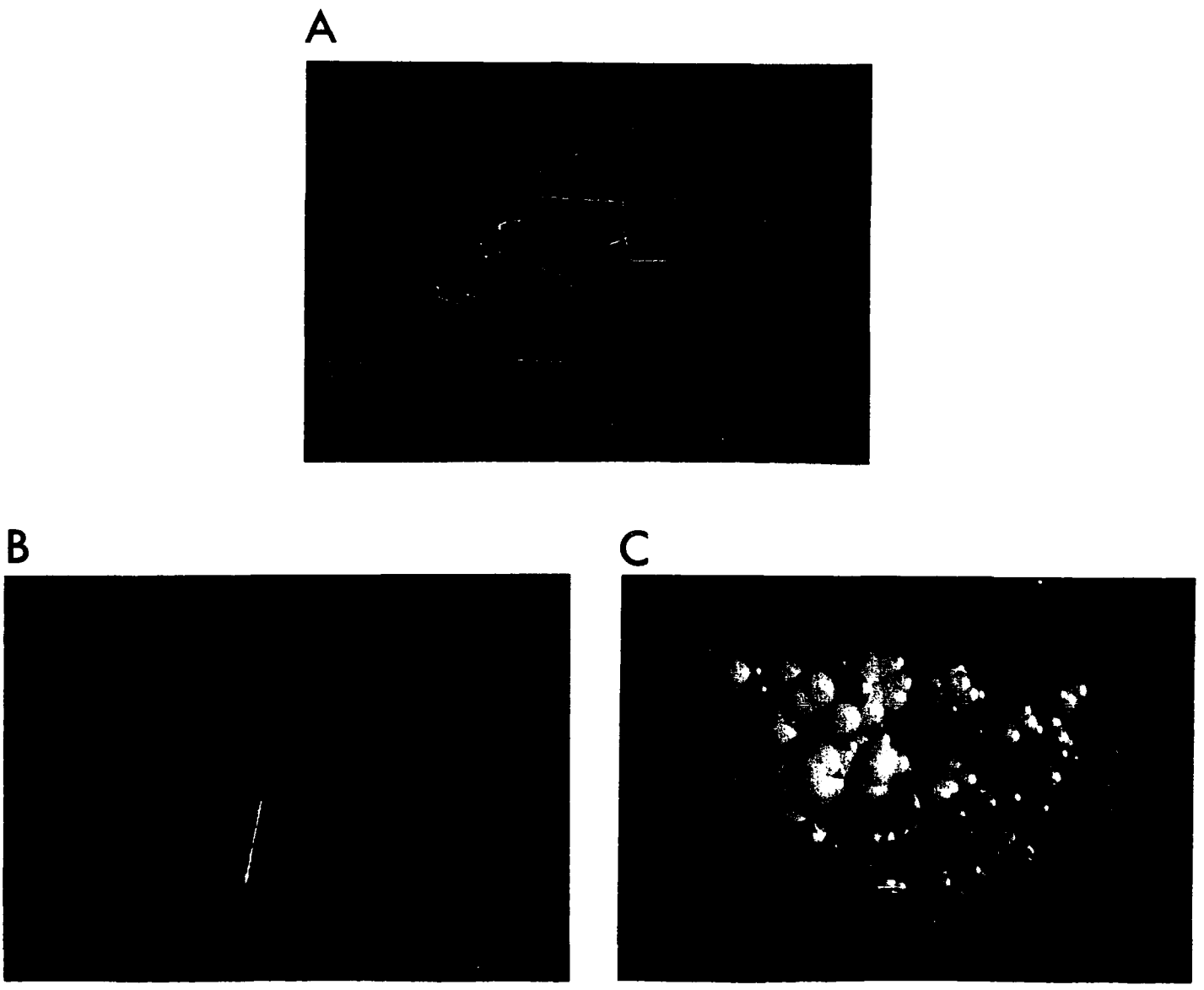

Figure 4: The structure of human defensin HNP-3 at 1.9 A. Two platinum derivatives were used to derive the structure (25). A. The main chain (green), the cystine disulfides (yellow), and the arginine side chains are depicted. B & C. All atoms of the dimer are shown in skeleton or space filling projection. The chain termini are oriented toward the top of the page, and the β hairpin at the bottom. The arrows indicate the vector of the hydrophobic moment. Atomic color code for B: carbon - yellow; amide N - pink; charged N - red; uncharged O - light blue; charged O - dark blue; S - charcoal.

PUBLICATIONS ACKNOWLEDGING ONR
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